Studies of the structure of bacteriophage λ *cro* protein in solution

Analysis of the circular dichroism data

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The secondary and tertiary structures of bacteriophage *cro* protein were studied by circular dichroism. The pH dependence of this structure was investigated: *cro* protein is stable over pH 4.5–10.5. At these pH-values *cro* protein contains ~35% α-helix, ~20% antiparallel β-structure and ~15% β-turn, while the remaining part of the protein molecule is in the irregular state. The secondary and tertiary structures of the protein are modified abruptly at more acid and more alkaline pH-values. The curves characterizing the secondary and tertiary structures of the protein are symbatic. The effect of Gu–HCl on the secondary and tertiary structures of *cro* protein at 22°C and pH 7.2 was studied also. The conformational transition occurs within 0.6–1.9 M Gu–HCl. The changes in the secondary and tertiary structures of the protein have a symbatic character. Thermal denaturation of *cro* protein was examined. A possible mechanism of the protein denaturation is discussed.

cro *Protein* *Repressor* *Secondary structure* *Denaturation* *Circular dichroism*

1. INTRODUCTION

Bacteriophage λ *cro* protein (Cro) is the smallest repressor protein characterized so far [1,2]. Cro is a basic protein (pI 9.9 [3]), containing 66 amino acid residues; its M_r is 7351. The primary structure of the protein [4] and the nucleotide sequence of the *cro* gene [5] have been determined. The small size of the monomer molecule, the presence of the three-dimensional structure in solution [6–9] in the absence of disulfide bonds make Cro an interesting and suitable model for studying not merely specific DNA–protein interaction, but also self-assembly of protein structures.

The three-dimensional structure of Cro was determined using X-ray diffraction analysis at a 2.8 Å resolution [10]. Each monomer consists of a 3-layer β-sheet and 3 α-helical regions. This structure has been predicted theoretically and independently based on the analysis of the protein primary structure [11]. As was shown using the techniques of high resolution PMR spectroscopy and CD, Cro also has a distinct tertiary structure in solution [6–9].

This paper presents the results of our studies on the secondary and tertiary structures of Cro using the CD method. These data are compared with the results of X-ray diffraction analysis, NMR studies and theoretical calculations. A possible mechanism of the protein denaturation is discussed.

2. MATERIALS AND METHODS

Cro was isolated from *Escherichia coli* strain pBR 294/214 [12] as in [8]. Protein solutions contained 0.2 M NaCl and, if the experiments were

Abbreviations: CD, circular dichroism; Cro, bacteriophage λ *cro* protein; NMR, nuclear magnetic resonance; Gu–HCl, guanidine chloride; UV, ultraviolet

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conducted at different temperatures, 10 mM Tris–HCl (pH 7.1). Concentrations of solutions were determined as in [9].

CD spectra were recorded by a MARK III S dichrograph (Jobin-Ivon). Measurements in the far UV region of the spectrum were done in 0.5 mm cells at ~0.5 mg protein/ml, whereas 1 cm cells and ~1 mg protein/ml were used in the near UV region. The obtained data were expressed as molar ellipticity calculated per averaged amino acid residue (θ). The mean residue molecular mass was calculated for the protein from its amino acid composition. It was equal to 111.6.

The secondary structure of the protein was determined from CD spectra recorded within 205–240 nm as in [13–20] using new protein-derived basic spectra obtained in [17–20] for 5 structures: α-helix, parallel and antiparallel β-structures; β-turns; and an irregular form.

3. RESULTS AND DISCUSSION

CD spectra were recorded for Cro in the far UV region (fig.1A) and in the near UV region (fig.1B) within pH 2–12.5 of the medium at 22°C. Analysis of the spectra has shown that, at neutral pH, Cro contains: ~35% α-helix; ~20% antiparallel β-structure; 0% of parallel β-structure; and ~15% of

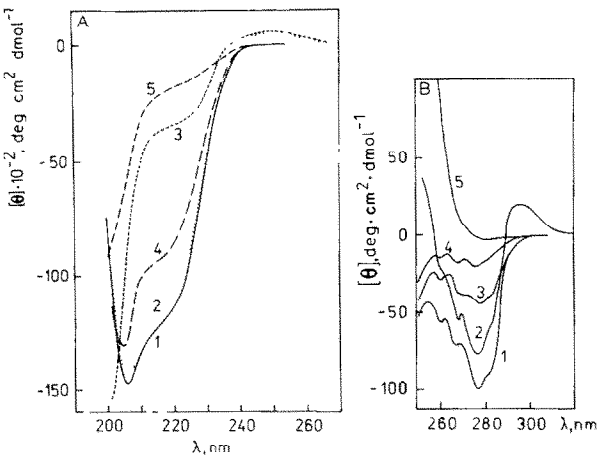

Fig.1. (A) The dependence of CD spectra for *cro* protein in the far UV region on the pH of the medium: (1) pH 7.15; (2) pH 10.91; (3) pH 12.28; (4) pH 4.03; (5) pH 2.06. (B) The dependence of 'aromatic' CD spectra for *cro* protein on the pH of the medium: (1) pH 6.18; (2) pH 10.15; (3) pH 3.60; (4) pH 2.41; (5) pH 11.93.

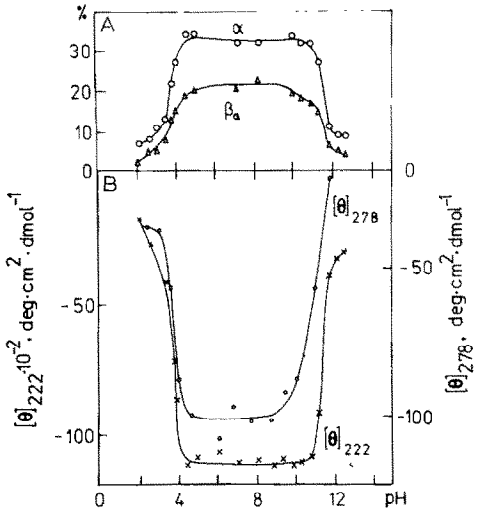

Fig.2. The percentage of α-helices and antiparallel β-structure as a function of the pH of the medium (A) and the pH dependence of the molar ellipticities at 222 and 278 nm (B).

β-turns; while the remaining part of the protein molecule is in the irregular state. The data obtained from the CD spectra are in good agreement with the results of X-ray diffraction analysis and theoretical calculations [10,11].

We have studied the pH dependence of the CD spectra in the far and near UV regions at 22°C. Fig.2 presents curves characterizing the secondary structure of the protein (the percentage of amino acid residues in the α-helices and in the regions of the antiparallel β-structure, the molar ellipticity $[\theta]_{222}$) and a curve characterizing the tertiary structure of the protein (the molar ellipticity $[\theta]_{278}$). Cro is stable within pH 4.5–10.5 (fig.2). The secondary and tertiary structures of the protein are modified abruptly at more acid and more alkaline pH values of the medium: the content of α-helical and antiparallel β-structural regions changes. The parallel β-structure is 0% at all pH values while that of β-turns remains constant and equal to ~15% throughout the entire pH range. The curves characterizing the secondary and tertiary structures of the protein are symbatic. However, at alkaline pH values, the tertiary structure of the protein begins to change slightly earlier than the secondary structure (see fig.2).

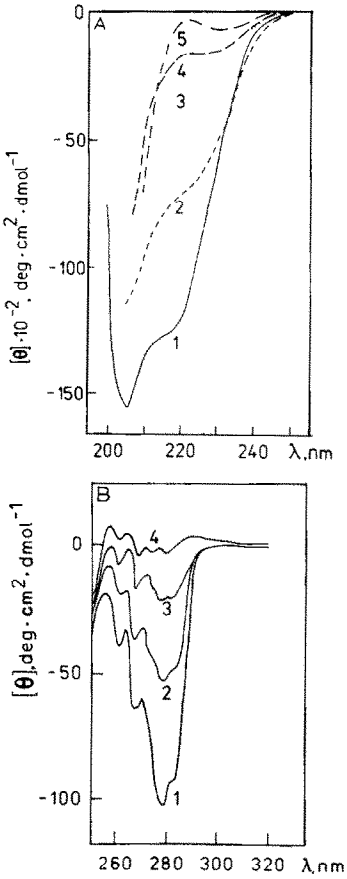

Fig.3. (A) The effect of Gu–HCl concentration on *cro* protein CD spectra in the far UV region: (1) 0 M Gu–HCl; (2) 1 M; (3) 1.25 M; (4) 1.5 M; (5) 3 M. (B) The effect of Gu–HCl concentration on *cro* protein CD spectra in the 'aromatic' region: (1) 0 M Gu–HCl; (2) 1 M; (3) 1.5 M; (4) 3 M.

Furthermore, we have studied the effect of guanidinium chloride on the secondary and tertiary structures of Cro at 22°C and pH 7.2. Fig.3A shows the pattern of CD spectra in the far UV region as a function of Gu–HCl concentration whereas fig.3B presents the dependence of 'aromatic' CD spectra. Fig.4 shows the effect of Gu–HCl concentration on the parameters characterizing the secondary structure of Cro (the percentage of α-helices and of the antiparallel β-structure and $[\theta]_{222}$) and its tertiary structure ($[\theta]_{278}$). The conformational transition, at 10% and 90% levels of ellipticity without Gu–HCl, occurs within 0.6–1.9 M Gu–HCl (fig.4). The changes in the secondary and tertiary structures of the protein have a symbatic character.

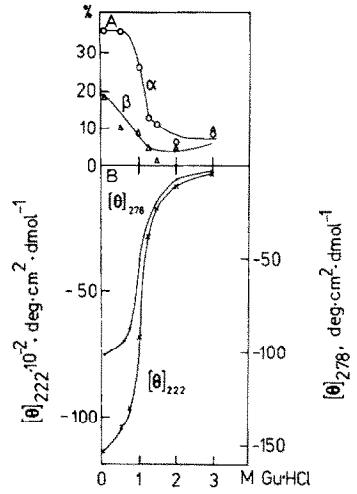

Fig.4. The percentage of α-helices and antiparallel β-structure as a function of [Gu–HCl] (A) and the effect of [Gu–HCl] on the molar ellipticities at 222 and 278 nm (B).

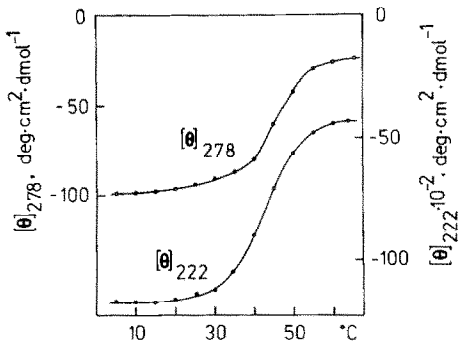

Fig.5. The temperature dependence of the molar ellipticities of *cro* protein solution.

We have also examined thermal denaturation of Cro. The curves for changes in the molar ellipticity at 222 and 278 nm over 5–65°C (fig.5) are indicative of an abrupt conformational transition. The curve characterizing the tertiary structure of Cro gives the half-transition temperature as ~45°C. The symbatic change of $[\theta]_{222}$ and $[\theta]_{278}$ shows that the secondary and tertiary structures of the protein are modified simultaneously as the temperature rises, which results in significant disorganization of the molecule. It is noteworthy that Cro denaturation is entirely reversible if the temperature of the solution is decreased slowly (~1°C/min).

A second conformational transition in Cro molecule near 20°C was found in [7] by the NMR

technique. However, the temperature dependences of the molar ellipticities in our work are indicative of merely one conformational transition. The weak monotonous change in the $[\theta]_{222}$ and $[\theta]_{278}$ at 5–30°C shows that conformational rearrangements are insignificant and that highly cooperative processes are absent. This is consistent with a minor change in the functional activity of Cro at 0–37°C, as was found in Cro-operator binding experiments [21,22].

These data are indicative of a high conformational stability of Cro in solution in the absence of denaturing agents.

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